

# Procedure & Checklist - Greater Than 10 kb Template Preparation Using AMPure® PB Beads

## Before You Begin

To perform this procedure, you must have the PacBio® DNA Template Prep Kit (verify you have the correct kit for your insert size) and have reviewed the *User Bulletin - Guidelines for Preparing 20 kb SMRTbell™ Templates*.

This procedure can be used to prepare greater than 10 kb libraries from 5 µg of sheared and concentrated DNA. If preparing larger amounts of DNA, scale all the reaction volumes proportionally (e.g., if the input amount of DNA is double the amount set forth in this procedure, then double all the reaction volumes listed in the tables).

If a BluePippin™ size selected system is not available or if the SMRTbell™ libraries are less than 500 ng, an alternative method using AMPure PB beads may be used to filter out most fragments in the 1 kb to 2 kb range. A more aggressive approach, using 0.40X AMPure PB beads (in the final step of the purification process) may also be used.

| Insert Size Target | Insert Size Range | Sheared and Concentrated DNA Amount | Ligation | DNA Damage Repair |
|--------------------|-------------------|-------------------------------------|----------|-------------------|
| 10 kb to 20 kb     | > 10 kb           | 5 µg                                | Blunt    | Required          |

## Fragment and Concentrate DNA

Before shearing your DNA sample, be sure to have assessed the quality and purified the sample according to the *User Bulletin - Guidelines for Preparing 20 kb SMRTbell™ Templates*.

Use a Covaris® g-TUBE® device to shear your DNA sample. The most up-to-date guidance on how to use the g-TUBE device, along with recommended centrifuges and centrifugation speeds, can be found in the g-TUBE device user manual available for download from the Covaris website. Depending upon the quality of your sample, approximately 20% to 50% sample loss is to be expected as a result of the shearing and concentration process. Therefore, be sure to have sufficient amounts of starting DNA in order to have at least 5 µg of sheared and concentrated DNA (140 ng/µL) for the Damage Repair reaction.

| STEP | ✓ | Concentrate DNA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Notes |
|------|---|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 1    |   | <p>Add <b>0.45X</b> volume of AMPure® PB magnetic beads.</p> <p>_____ µL of sample X <b>0.45X</b> = _____ µL of beads</p> <p>Note that the beads must be brought to room temperature and all AMPure PB bead purification steps should be performed at room temperature.</p> <p>Before using, mix the bead reagent well until the solution appears homogenous. Pipette the reagent slowly since the bead mixture is viscous and precise volumes are critical to the purification process.</p> <p>Consistent and efficient recovery of your sample is critical to successful SMRTbell™ template preparation. If using this protocol for the first time, we strongly recommend that you process a control sample first. Using the DNA shearing methods and subsequent AMPure PB bead purification steps described below, you should recover approximately <b>50%-80%</b> of your input DNA (by mass). Typical yields, from pre-purified DNA (where smaller fragments are already eliminated as a result of the shearing process) are between 80-100%.</p> |       |
| 2    |   | Mix the bead/DNA solution thoroughly.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |       |
| 3    |   | Quickly spin down the tube (for 1 second) to collect the beads.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       |
| 4    |   | <p>Allow the DNA to bind to beads by shaking in a VWR® vortex mixer at 2000 rpm for 10 minutes at room temperature. Note that the bead/DNA mixing is critical to yield. After vortexing, the bead/DNA mixture should appear homogenous.</p> <p>We recommend using a VWR vortex mixer with a foam microtube attachment (see the <i>Guide's</i> Overview section for part numbers). If using other instrumentation, ensure that the mixing is equally vigorous. Failure to thoroughly mix the DNA with the bead reagent will result in inefficient DNA binding and reduced sample recoveries.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                        |       |
| 5    |   | Spin down the tube (for 1 second) to collect beads.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |       |
| 6    |   | Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear. The actual time required to collect the beads to the side depends on the volume of beads added.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |       |
| 7    |   | <p>With the tube still on the magnetic bead rack, slowly pipette off cleared supernatant and save in another tube. Avoid disturbing the bead pellet.</p> <p>If the DNA is not recovered at the end of this Procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |       |
| 8    |   | <p>Wash beads with freshly prepared 70% ethanol.</p> <p>Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days.</p> <ul style="list-style-type: none"> <li>– Do not remove the tube from the magnetic rack.</li> <li>– Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. Let the tube sit for 30 seconds.</li> <li>– Do not disturb the bead pellet.</li> <li>– After 30 seconds, pipette and discard the 70% ethanol.</li> </ul>                                                                                                                                                                                                                                                                                                                                      |       |
| 9    |   | Repeat <a href="#">step 8</a> above.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |       |

| STEP | ✓ | Concentrate DNA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Notes |
|------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 10   |   | <p>Remove residual 70% ethanol and dry the bead pellet.</p> <ul style="list-style-type: none"> <li>– Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube.</li> <li>– Place the tube back on magnetic bead rack.</li> <li>– Pipette off any remaining 70% ethanol.</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |       |
| 11   |   | Check for any remaining droplets in the tube. If droplets are present, repeat <a href="#">step 10</a> .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |       |
| 12   |   | Remove the tube from the magnetic bead rack and allow beads to air-dry (with the tube caps open) for 30 to 60 seconds.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |       |
| 13   |   | <p>Calculate appropriate volume of Elution Buffer.</p> <p>_____ ng X <b>0.5</b> / (_____ng/μL) = _____ μL of Elution Buffer needed</p> <p>The minimum DNA concentration required to proceed to the next step (End-Repair) is <b>140 ng/μL</b> with preferred mass of at least <b>5 μg</b>.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |       |
| 14   |   | <p>Add the Pacific Biosciences® Elution Buffer volume (calculated in <a href="#">step 13</a> above) to your beads.</p> <ul style="list-style-type: none"> <li>– Thoroughly resuspend beads by vortexing for 1 minute at 2000 rpm. If the beads appear over-dried or cracked, let the Elution Buffer sit on the beads for 2 to 3 minutes then vortex again.</li> <li>– Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack.</li> <li>– Perform concentration measurements. Verify your DNA concentration using a Nanodrop® or Qubit® quantitation platform. If the DNA concentration is estimated to be equal to or below <b>12 ng/μL</b>, a Qubit system reading is required. When performing a Qubit system reading, ensure that your sample is within the range of the Qubit kit you are using. For proper concentration calculations, incorporate the dilution factor (used when diluting your sample) to be within range of the Qubit kit and the dilution factor when diluting your sample with the working solution. The latter part of this dilution factor can be calculated automatically by the Qubit system.</li> <li>– Discard the beads.</li> </ul> |       |
| 15   |   | <p>Perform qualitative and quantitative analysis using a Bioanalyzer® instrument. Note that the Bioanalyzer instrument has different kits in its offering and the appropriate kit, based on insert size, should be used.</p> <p>Dilute the samples appropriately before loading on the Bioanalyzer chip so that the DNA concentration loaded falls well within the detectable minimum and maximum range of the assay. Refer to Agilent Technologies' guides for specific information on the range of the specific kit you might be using.</p> <p>Note that typical yield, at this point of the process (i.e. post-shearing and after one <b>0.45X</b> AMPure PB bead purification), is approximately <b>50%-80%</b>.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |
| 16   |   | The sheared DNA can be stored for up to 24 hours at 4°C or at -20°C for longer duration.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |       |
| 17   |   | Actual recovery per μL and total available sample material: _____                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |       |

## Repair DNA Damage

Use the following table to repair any DNA damage. If preparing larger amounts of DNA, scale the reaction volumes accordingly (i.e., for 10 µg of DNA scale the total volume to 100 µL). Do not exceed 100 ng/µL of DNA in the final reaction.

1. In a LoBind microcentrifuge tube, add the following reagents:

| Reagent                  | Tube Cap Color                                                                    | Stock Conc. | Volume                       | Final Conc. | ✓ | Notes |
|--------------------------|-----------------------------------------------------------------------------------|-------------|------------------------------|-------------|---|-------|
| Sheared DNA              | –                                                                                 |             | ___ µL for 5.0 µg            | –           |   |       |
| DNA Damage Repair Buffer |  | 10 X        | 5.0 µL                       | 1 X         |   |       |
| NAD <sup>+</sup>         |  | 100 X       | 0.5 µL                       | 1 X         |   |       |
| ATP high                 |  | 10 mM       | 5.0 µL                       | 1 mM        |   |       |
| dNTP                     |  | 10 mM       | 0.5 µL                       | 0.1 mM      |   |       |
| DNA Damage Repair Mix    |  |             | 2.0 µL                       |             |   |       |
| H <sub>2</sub> O         | –                                                                                 |             | ___ µL to adjust to 50.0* µL | –           |   |       |
| Total Volume             |                                                                                   |             | 50.0 µL                      | –           |   |       |

\*To determine the correct amount of H<sub>2</sub>O to add, use your actual DNA amount noted in the Notes column.

2. Mix the reaction well by pipetting or flicking the tube.
3. Spin down contents of tube with a quick spin in a microfuge.
4. Incubate at 37°C for 20 minutes, then return the reaction to 4°C for 1 to 5 minutes.

## Repair Ends

Use the following table to prepare your reaction then purify the DNA.

| Reagent               | Tube Cap Color                                                                      | Stock Conc. | Volume  | Final Conc. | ✓ | Notes |
|-----------------------|-------------------------------------------------------------------------------------|-------------|---------|-------------|---|-------|
| DNA (Damage Repaired) | –                                                                                   |             | 50 µL   | –           |   |       |
| End Repair Mix        |  | 20 X        | 2.5 µL  | 1X          |   |       |
| Total Volume          |                                                                                     |             | 52.5 µL | –           |   |       |

1. Mix the reaction well by pipetting or flicking the tube.
2. Spin down contents of tube with a quick spin in a microfuge.
3. Incubate at 25°C for 5 minutes, return the reaction to 4°C.

| STEP | ✓ | Purify DNA                                                                                                                                                                                                                                                                                                                                                    | Notes |
|------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 1    |   | Add <b>0.45X</b> volume of AMPure PB beads to the End-Repair reaction. (For detailed instructions on AMPure PB bead purification, see the Concentrate DNA section).                                                                                                                                                                                           |       |
| 2    |   | Mix the bead/DNA solution thoroughly.                                                                                                                                                                                                                                                                                                                         |       |
| 3    |   | Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.                                                                                                                                                                                                                                                                          |       |
| 4    |   | Allow the DNA to bind to beads by shaking in a VWR vortex mixer at 2000 rpm for 10 minutes at room temperature.                                                                                                                                                                                                                                               |       |
| 5    |   | Spin down the tube (for 1 second) to collect beads.                                                                                                                                                                                                                                                                                                           |       |
| 6    |   | Place the tube in a magnetic bead rack to collect the beads to the side of the tube.                                                                                                                                                                                                                                                                          |       |
| 7    |   | Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet.                                                                                                                                                                                                                                                          |       |
| 8    |   | Wash beads with freshly prepared 70% ethanol.                                                                                                                                                                                                                                                                                                                 |       |
| 9    |   | Repeat <a href="#">step 8</a> above.                                                                                                                                                                                                                                                                                                                          |       |
| 10   |   | Remove residual 70% ethanol and dry the bead pellet. <ul style="list-style-type: none"> <li>– Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube.</li> <li>– Place the tube back on magnetic bead rack.</li> <li>– Pipette off any remaining 70% ethanol.</li> </ul> |       |
| 11   |   | Check for any remaining droplets in the tube. If droplets are present, repeat <a href="#">step 10</a> .                                                                                                                                                                                                                                                       |       |
| 12   |   | Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.                                                                                                                                                                                                                                            |       |
| 13   |   | Elute the DNA off the beads in <b>30 µL</b> Elution Buffer. Mix until homogenous, then vortex for 1 minute at 2000 rpm.                                                                                                                                                                                                                                       |       |
| 14   |   | Optional: Verify your DNA amount and concentration using a Nanodrop or Qubit quantitation platform, as appropriate.                                                                                                                                                                                                                                           |       |
| 15   |   | Optional: Perform qualitative and quantitative analysis using a Bioanalyzer instrument with the DNA 12000 Kit. Note that typical yield at this point of the process (following End-Repair and one <b>0.45X</b> AMPure PB bead purification) is approximately between <b>80-100%</b> of the total starting material.                                           |       |
| 16   |   | The End-Repaired DNA can be stored overnight at 4°C or at -20°C for longer duration.                                                                                                                                                                                                                                                                          |       |
| 17   |   | Actual recovery per µL and total available sample material: _____                                                                                                                                                                                                                                                                                             |       |

## Prepare Blunt Ligation Reaction

Use the following table to prepare your blunt ligation reaction:

1. In a LoBind microcentrifuge tube (on ice), add the following reagents in the order shown. Note that you can add water to achieve the desired DNA volume. If preparing a Master Mix, ensure that the adapter is NOT mixed with the ligase prior to introduction of the inserts. Add the adapter to the well with the DNA. All other components, including the ligase, should be added to the Master Mix.

| Reagent                               | Tube Cap Color                                                                    | Stock Conc. | Volume                    | Final Conc. | ✓ | Notes |
|---------------------------------------|-----------------------------------------------------------------------------------|-------------|---------------------------|-------------|---|-------|
| DNA (End Repaired)                    | –                                                                                 |             | 29.0 µL to 30.0 µL        |             |   |       |
| <b>Annealed</b> Blunt Adapter (20 µM) |  | 20 µM       | 1.0 µL                    | 0.5 µM      |   |       |
| Mix before proceeding                 |                                                                                   |             |                           |             |   |       |
| Template Prep Buffer                  |  | 10 X        | 4.0 µL                    | 1X          |   |       |
| ATP low                               |  | 1 mM        | 2.0 µL                    | 0.05 mM     |   |       |
| Mix before proceeding                 |                                                                                   |             |                           |             |   |       |
| Ligase                                |  | 30 U/µL     | 1.0 µL                    | 0.75 U/µL   |   |       |
| H <sub>2</sub> O                      | –                                                                                 | –           | — µL to adjust to 40.0 µL | –           |   |       |
| Total Volume                          | –                                                                                 | –           | 40.0 µL                   | –           |   |       |

2. Mix the reaction well by pipetting or flicking the tube.
3. Spin down contents of tube with a quick spin in a microfuge.
4. Incubate at 25°C overnight.
5. Incubate at 65°C for 10 minutes to inactivate the ligase, then return the reaction to 4°C. You must proceed with adding exonuclease after this step.

Add exonuclease to remove failed ligation products.

| Reagent                        | Tube Cap Color                                                                      | Stock Conc. | ✓ | Volume |
|--------------------------------|-------------------------------------------------------------------------------------|-------------|---|--------|
| Ligated DNA                    |                                                                                     |             |   | 40 µL  |
| Mix reaction well by pipetting |                                                                                     |             |   |        |
| ExoIII                         |  | 100.0 U/µL  |   | 1.0 µL |
| ExoVII                         |  | 10.0 U/µL   |   | 1.0 µL |
| Total Volume                   |                                                                                     |             |   | 42 µL  |

1. Spin down contents of tube with a quick spin in a microfuge.
2. Incubate at 37°C for 1 hour, then return the reaction to 4°C. You must proceed with purification after this step.

## Purify SMRTbell™ Templates

There are 3 purification steps, 2 using 0.45X volume of AMPure PB beads and a final purification of 0.40X volumes of AMPure PB beads

| STEP | ✓ | Purify SMRTbell™ Templates                                                                                                                                                                                                                                                                                                                                    | Notes |
|------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 1    |   | Add <b>0.45X</b> volume of AMPure PB beads to the exonuclease-treated reaction. (For detailed instructions on AMPure PB bead purification, see the Concentrate DNA section).                                                                                                                                                                                  |       |
| 2    |   | Mix the bead/DNA solution thoroughly.                                                                                                                                                                                                                                                                                                                         |       |
| 3    |   | Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.                                                                                                                                                                                                                                                                          |       |
| 4    |   | Allow the DNA to bind to beads by shaking in a VWR vortex mixer at 2000 rpm for 10 minutes at room temperature.                                                                                                                                                                                                                                               |       |
| 5    |   | Spin down the tube (for 1 second) to collect beads.                                                                                                                                                                                                                                                                                                           |       |
| 6    |   | Place the tube in a magnetic bead rack to collect the beads to the side of the tube.                                                                                                                                                                                                                                                                          |       |
| 7    |   | Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet.                                                                                                                                                                                                                                                          |       |
| 8    |   | Wash beads with freshly prepared 70% ethanol.                                                                                                                                                                                                                                                                                                                 |       |
| 9    |   | Repeat <a href="#">step 8</a> above.                                                                                                                                                                                                                                                                                                                          |       |
| 10   |   | Remove residual 70% ethanol and dry the bead pellet. <ul style="list-style-type: none"> <li>– Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube.</li> <li>– Place the tube back on magnetic bead rack.</li> <li>– Pipette off any remaining 70% ethanol.</li> </ul> |       |
| 11   |   | Check for any remaining droplets in the tube. If droplets are present, repeat <a href="#">step 10</a> .                                                                                                                                                                                                                                                       |       |
| 12   |   | Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.                                                                                                                                                                                                                                            |       |
| 13   |   | Elute the DNA off the beads in <b>50 µL</b> of Elution Buffer. Vortex for 1 minute at 2000 rpm.                                                                                                                                                                                                                                                               |       |
| 14   |   | The eluted DNA in <b>50 µL</b> Elution Buffer should be taken into the second <b>0.45X</b> AMPure PB bead purification step.                                                                                                                                                                                                                                  |       |

| STEP | ✓ | Purify SMRTbell™ Templates - Second Purification                                                                                                                                                                                                                                                                                                              | Notes |
|------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 1    |   | Add <b>22.5 µL (0.45X)</b> volume) of AMPure PB beads to the <b>50 µL</b> of eluted DNA from the first AMPure PB bead purification step above. (For detailed instructions on AMPure PB bead purification, see the Concentrate DNA section)                                                                                                                    |       |
| 2    |   | Mix the bead/DNA solution thoroughly.                                                                                                                                                                                                                                                                                                                         |       |
| 3    |   | Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.                                                                                                                                                                                                                                                                          |       |
| 4    |   | Allow the DNA to bind to beads by shaking in a VWR vortex mixer at 2000 rpm for 10 minutes at room temperature.                                                                                                                                                                                                                                               |       |
| 5    |   | Spin down the tube (for 1 second) to collect beads.                                                                                                                                                                                                                                                                                                           |       |
| 6    |   | Place the tube in a magnetic bead rack to collect the beads to the side of the tube.                                                                                                                                                                                                                                                                          |       |
| 7    |   | Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet.                                                                                                                                                                                                                                                          |       |
| 8    |   | Wash beads with freshly prepared 70% ethanol.                                                                                                                                                                                                                                                                                                                 |       |
| 9    |   | Repeat <a href="#">step 8</a> above.                                                                                                                                                                                                                                                                                                                          |       |
| 10   |   | Remove residual 70% ethanol and dry the bead pellet. <ul style="list-style-type: none"> <li>– Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube.</li> <li>– Place the tube back on magnetic bead rack.</li> <li>– Pipette off any remaining 70% ethanol.</li> </ul> |       |
| 11   |   | Check for any remaining droplets in the tube. If droplets are present, repeat <a href="#">step 10</a> .                                                                                                                                                                                                                                                       |       |
| 12   |   | Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.                                                                                                                                                                                                                                            |       |
| 13   |   | Elute the DNA off the beads in <b>100 µL</b> of Elution Buffer. Vortex for 1 minute at 2000 rpm.                                                                                                                                                                                                                                                              |       |
| 14   |   | Verify your DNA amount and concentration with either a Nanodrop or Qubit quantitation platform reading.                                                                                                                                                                                                                                                       |       |

| STEP | ✓ | Purify SMRTbell™ Templates - Third Purification                                                                                                                                                                                                                                                                                                                                                                     | Notes |
|------|---|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| 1    |   | Add <b>40 µL</b> or ( <b>0.40X</b> ) of AMPure PB beads to the <b>100 µL</b> of eluted DNA. Note that for <b>0.40X</b> volumes, it is critical to accurately pipet the desired volume of AMPure PB bead solution; there is a steep drop-off in recovery for concentrations <b>&lt;0.40X</b> .                                                                                                                       |       |
| 2    |   | Mix the bead/DNA solution thoroughly.                                                                                                                                                                                                                                                                                                                                                                               |       |
| 3    |   | Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.                                                                                                                                                                                                                                                                                                                                |       |
| 4    |   | Allow the DNA to bind to beads by shaking in a VWR vortex mixer at 2000 rpm for 10 minutes at room temperature.                                                                                                                                                                                                                                                                                                     |       |
| 5    |   | Spin down the tube (for 1 second) to collect beads.                                                                                                                                                                                                                                                                                                                                                                 |       |
| 6    |   | Place the tube in a magnetic bead rack to collect the beads to the side of the tube.                                                                                                                                                                                                                                                                                                                                |       |
| 7    |   | Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet.<br><br>Note: It is especially important to save the supernatant for <b>0.40X</b> volumes of AMPure PB purification steps. In case of low recovery, perform a <b>1X</b> AMPure PB purification step to recover the DNA.                                                                                         |       |
| 8    |   | Wash beads with freshly prepared 70% ethanol.                                                                                                                                                                                                                                                                                                                                                                       |       |
| 9    |   | Repeat <b>step 8</b> above.                                                                                                                                                                                                                                                                                                                                                                                         |       |
| 10   |   | Remove residual 70% ethanol and dry the bead pellet.<br><ul style="list-style-type: none"> <li>– Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube.</li> <li>– Place the tube back on magnetic bead rack.</li> <li>– Pipette off any remaining 70% ethanol.</li> </ul>                                                    |       |
| 11   |   | Check for any remaining droplets in the tube. If droplets are present, repeat <b>step 10</b> .                                                                                                                                                                                                                                                                                                                      |       |
| 12   |   | Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.                                                                                                                                                                                                                                                                                                  |       |
| 13   |   | Elute the DNA off the beads in <b>10 µL</b> of Elution Buffer. Vortex for 1 minute at 2000 rpm.                                                                                                                                                                                                                                                                                                                     |       |
| 14   |   | Verify your DNA amount and concentration with either a Nanodrop or Qubit quantitation platform reading. For general library yield expect 20% total yield from the Damage Repair input. If your yield concentration is below 12 ng/µL, use the Qubit system for quantitation.<br><br>To estimate your final concentration:<br>( ____ ng of DNA going into Damage Repair X 0.2) / ____ of Elution Buffer = ____ ng/µL |       |
| 15   |   | Perform qualitative and quantitative analysis using a Bioanalyzer instrument. Note that typical DNA yield, at this point of the process (at the end of library preparation) is between approximately <b>5-20%</b> of the total starting DNA amount.                                                                                                                                                                 |       |

## Anneal and Bind SMRTbell™ Templates

Use the Binding Calculator in RS Remote to anneal sequencing primer at 0.833 nM concentration and bind polymerase at 0.500 nM concentration.

For the fields listed below, enter or select the following:

- Average insert size, enter: the estimated size provided by your Bioanalyzer instrument
- Select Magnetic Beads
- Small scale reaction
- Optional > Concentration On Plate, select Default (for 0.025 nM) or Custom and enter your chosen loading concentration

For more information about using the Binding Calculator, see the *Pacific Biosciences Template Preparation and Sequencing Guide*.

## Prepare for MagBead Loading

Optimal loading of > 10 kb templates is typically achieved at 0.025 nM on-plate concentration. A loading titration starting with 0.025 nM on-plate concentrations is highly recommended.

For efficient binding to Magnetic Beads, bound complexes (at 0.500 nM template concentration) must be diluted in the appropriate ratio of MagBead Binding Buffer and MagBead Wash Buffer. Refer to the Calculator for the appropriate dilution recommendations.

## DNA Control Complex Dilution

If you will be using the PacBio DNA Control Complex, dilute the DNA Control Complex according to the volumes and instructions specified in the Calculator.

## Anneal and Bind SMRTbell™ Templates

You must have the PacBio DNA/Polymerase Kit for this step. To anneal sequencing primer and bind polymerase to SMRTbell templates, see the Calculator in RS Remote. For more information about using the calculator, see the *Pacific Biosciences Template Preparation and Sequencing Guide*.

## Sequence

To prepare for sequencing on the instrument, refer to the *RS Remote Online Help* system or *Pacific Biosciences Software Getting Started Guide* for more information. Follow the touchscreen UI to start your run. Note that you must have a DNA Sequencing Kit and SMRT® Cells for standard sequencing.